REMARKS

Reconsideration and withdrawal of the restriction requirement are respectfully requested in view of the remarks herewith.

Group IV, the expression product, is elected with traverse; and, to complete that election, a particular species – erythropoietin – is elected. Claims 96-116 are presented, with these claims representing subject matter that Applicants particularly want searched and examined in this application, especially claim 96.

However, Applicants also traverse the restriction requirement, particularly as to Groups III, II and I, as it is verily believed that the search and examination of the subject matter of claims 96-116 will be co-extensive with searching and examining Groups III, II and I; and, the materials previously submitted, e.g., PCT search, shows that the search and examination of all of the claims of Groups I-IV can be done without any undue or serious burden.

It is respectfully pointed out that the amendments herein include an amendment to the lineage of the application to claim priority to an earlier co-pending patent application, and text from the previously co-pending and now issued U.S. Patent that was incorporated into the present application. Thus, the present application is now a continuation-in-part of application serial number 09/169,178, now patent number 6,103,526. As the present application was filed on January 18, 2000 and incorporated by reference USSN 09/169,178, and 09/169,178 issued on August 15, 2000, the two applications were co-pending and the text of USSN 09/169,178 is set forth in the instant application, such that it is believed no barrier exists to the amendments to the specification.

If any petition is required for amending the lineage of the instant application, please consider this paper to be a petition for amending the lineage of the application. Continuing status as to USSN 09/169,178 was inadvertently not claimed earlier.

Any fee for this petition, for a small entity, or any overpayment in such a fee, may be charged or credited to Deposit Account No. 50-0320.

Continuing status as to USSN 09/169,178 was inadvertently not claimed earlier because there was, prior to the present Office Action, no restriction of the claims specifically to expression products as in Group IV of the instant Office Action.

Such a restriction requirement, it is respectfully submitted, could have further precipitated an election of species or further restriction requirement as to the nature of the

expressed product, particularly in view of the text set forth at page 1 of the instant application, which teaches that the exogenous DNA and hence the expressed product, can be as in any or all of those stated in the applications cited at page 1.

Upon making the election and appreciating that a particular expressed product should be stated in the claims, and that that expressed product should be erythropoietin, it was appreciated that a claim such as claim 96 can be advanced. Accordingly, adding a lineage to USSN 09/169,027 and text therefrom was appreciated and is herein done.

No new matter is added; and, a new Declaration and Power of Attorney will be provided in due course. Any necessary amendment to the inventive entity will be attended to; and, an Information Disclosure Statement providing a copy of the documents cited in the text added by the Amendment is also intended.

It is respectfully requested that the application be accorded a lineage to USSN 09/169,027.

The October 17, 2002 Office Action called for restriction from among the following:

Group I: claims 43-49 and 85, drawn to a method to culture cells in a bioreactor, classified under class 435, subclass 325 or 41;

Group II: claims 50-72 and 74-85, drawn to a second method of cultivating cells in a bioreactor via recycling through a dialysis means, classified under class 435, subclass 383;

Group III: claims 73, 86-89 and 91-95, drawn to a method to produce an expression product, classified under class 435, subclass 358; and

Group IV: claim 90, drawn to an expressed product, classified under class 435, subclass 243.

As stated above, Group IV is elected, with traverse.

The amendments herein include the addition of new claims 96-116, directed to the expression product erythropoietin. It is verily believed that these new claims fall within Group IV, such that Group IV comprises claims 90, and 96-116.

The MPEP lists two criteria for a proper restriction requirement. First, the invention must be independent or distinct. MPEP § 803. Second, searching the additional invention must constitute an undue burden on the examiner if restriction is not required. *Id.* The MPEP directs the examiner to search and examine an entire application "[i]f the search and examination of an

entire application can be made without serious burden, ... even though it includes claims to distinct or independent inventions." *Id.*

The Office Action states that the "inventions are distinct ... because ... [t]he methods of Groups I-II are each directed to different inventions." Office Action at 3. Additionally, "different inventions disclosed in the claims encompassing ...Groups I-III are each methods to cultivate cells or to produce a product, however, each one of them will not be simultaneously applicable." Office Action at 3.

Further, the Office Action states that "Groups III and IV are related to each other as product and process to make the said product ...[and] are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another an materially different process." Office Action at 4.

In the instant case, the Office Action alleged that Groups III and IV satisfy both of (1) and (2) above.

It is again respectfully submitted that MPEP § 803 directs the examiner to search and examine an entire application "[i]f the search and examination of an entire application can be made without serious burden, ... even though it includes claims to distinct or independent inventions."

The Office Action maintains that the Groups are independent inventions, but, it is respectfully submitted, does not provide an adequate demonstration that the examination of more than one Group would present an undue burden on the Examiner.

Specifically, the claims of all Groups are classifed in class 435, such that there will be overlap between the searches.

Further, Groups III and IV are so closely related that any search of an expressed product will necessarily encompass the method of expression, and *vice versa*.

Consequently, it is respectfully submitted that the restriction requirement should be reconsidered and withdrawn and that Groups I-IV be examiner together, or at the least, that Groups III and IV should be rejoined, such that Applicants elect claims 73, and 86-116; and, if there are any additional requisite elections of species, the vector system is a baculovirus expression system and the expresssed protein is erythropoeitin, e.g., as set forth in claim 96.

In addition, it is noted that the February 26, 2002 Communication provided documents showing that in International Prosecution, all of the claims of Groups I to IV were searched and examined together, further showing that there is no undue or serious burden in searching and examining Groups I to IV together in this application; and, the March 18, 2002 Office Action had claims 43-95 in one Group, such that the PTO in this prosecution recognized that all of claim 43-95 (Groups I to IV) can be searched and examined together in this application. Accordingly, it is respectfully submitted that the prosecution to date and the International Prosecution of the corresponding PCT application demonstrates that there is no undue or serious burden in searching and examining all of Groups I to IV in this application, and mandate against restriction as set forth in the instant Office Action.

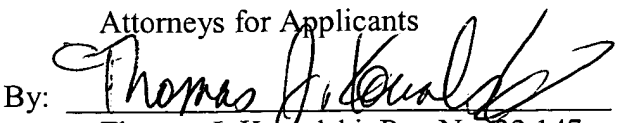
In summary, enforcing the present restriction requirement would result in inefficiencies and unnecessary expenditures by both the Applicants and the PTO, as well as extreme prejudice to Applicants (particularly in view of GATT, whereby a shortened patent term may result in any divisional applications filed). Restriction has not been shown to be proper, especially since it has been shown that the suggested alternate utility for the apparatus is improper, and since the requisite showing of serious burden has not been made. Indeed, the search and examination of each Group would be likely to be co-extensive and, in any event, would involve such interrelated art that the search and examination of the entire application can be made without undue burden on the Examiner, especially as the USPTO has previously searched and examined the entire application while preparing the IPER in the corresponding International Application. All of the preceding, therefore, mitigate against restriction.

CONCLUSION

In view of the amendments and remarks herein, reconsideration and withdrawal of the restriction requirement are respectfully requested. Early and favorable consideration of the application on the merits, and Allowance of the application are earnestly solicited.

Respectfully submitted,

FROMMER LAWRENCE & HAUG LLP
Attorneys for Applicants

By: 

Thomas J. Kowalski, Reg No. 32,147
Angela M. Nigro, Reg. No. 51,107
Tel 212-588-0800, Fax 212-588-0500

APPENDIX: MARKED VERSION OF AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

IN THE SPECIFICATION

Kindly amend the specification, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

Page 1, between the first and second paragraphs (under the heading "Related Applications") please insert:

--This application is also a continuation-in-part of U.S. application Serial No. 09/169,178 filed October 8, 1998 and issued as U.S. patent No. 6,103,526 on August 15, 2000.--

Page 54, after the first paragraph, and before the three centered stars above the second paragraph, please add:

--**EXAMPLE 9 - Human Erythropoietin**

The sequence of human erythropoietin (EPO) is available from GenBank (accession no. X02157). The human EPO gene isolated from a genomic library in bacteriophage Lambda EMBL-3 was used as template to amplify EPO coding sequences by PCR. A construct was made in which EPO's natural signal peptide was replaced by a baculovirus signal peptide. A 5' PCR primer was made that began at the N-terminal residue of the mature peptide. A 3' primer was designed to terminate after the natural stop codon of the EPO open reading frame. After PCR amplification, the resulting EPO gene fragment was inserted into the pMGS12 baculovirus transfer plasmid using standard procedures (Sambrook, J, Fritsch, E. F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The resulting transfer plasmid contained the coding region from EPO downstream of the polyhedrin promoter, flanked by AcNPV DNA from the EcoRI "I" fragment (Summers and Smith. A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, May 1987, Texas A&M University). Confirmation of the correct EPO coding sequence (Jacobs et al. Nature 313 806-10 (1985)) was determined by DNA sequence analysis.

Genomic baculovirus DNA and the transfer plasmids containing the EPO gene were mixed, co-precipitated with calcium chloride, and Sf900+ cells (ATCC CRL-12579, deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas,

Va. 20110-2009, under the terms of the Budapest Treaty, under ATCC Designation on Sep. 18, 1998) were transfected as described (Summers and Smith 1987, supra). Recombinant viruses were identified by plaque morphology and several were further plaque purified. Recombinant viruses capable of expressing EPO in infected Sf900+ cellswere identified and used as baculovirus expression vectors to produce recombinant EPO in Sf900+ cells.

Sf900+ cells, at a cell density of 1.5 $x10^6$ cells/ml are infected with the baculovirus expression vector containing the EPO gene at an MOI of 1.0. Sf900+ cells are harvested by centrifugation 72 hours post infection. The cell pellet is discarded and the supernatant containing secreted recombinant EPO ("rEPO") is stored at 4.degree. C. for further processing.

Product purification follows centrifugation, filtration and chromatographic procedures analogous to those presented for influenza virus hemagglutinin (U.S. Pat. No. 5,762,939 and allowed U.S. application Ser. No. 08/453,848, incorporated herein by reference). Thus, EPO can be obtained which is purified to substantial homogeneity or to at least 95% purity. With respect to EPO, DNA encoding EPO and substantial homogeneity of EPO, reference is also made to Lin, U.S. Pat. Nos. 4,703,008, 5,441,868, 5,574,933, 5,618,698, 5,621,080, and 5,756,349. In addition, reference is also made to Wojchowski et al., "Active Human Erythropoietin Expressed in Insect Cells, Using a Baculovirus Vector: A Role For N-Linked Oligosaccharide", Biochimica et Biophysica Acta 910:224-32 (1987), Quelle et al., "High-Level Expression and Purification of a Recombinant Human Erythropoietin Produced Using a Baculovirus Vector", Blood, 74(2):652-57 (1989), Quelle et al., "Phosphorylatable and Epitope-Tagged Human Erythropoietins: Utility and Purification of Native Baculovirus-Derived Forms", Protein Expression and Purification 3:461-69 (1992), and U.S. Pat. Nos. 5,322,837 and 4,677,195. In contrast to any prior EPO from baculovirus expression, EPO in accordance with the present invention can be purified to at least 95% purity or to substantial homogeneity; and, the EPO in accordance with the present invention is produced in relatively high amounts, is glycosylated and secreted, and has physical and biological properties as follows: 25 kD, secreted monomers; stimulates erythropoiesis. stimulates erythropoiesis.

As a particular purification procedure, centrifuged culture supernatant containing rEPO is pH adjusted to pH 8.0 with Tris-base. Proteinatious and non-proteinatious materials bind to precipitating salts, mainly calcium hydroxide, and are removed by centrifugation while rEPO

remains in the supernatant. The resulting rEPO containing supernatant is diafiltered into 10 mM Tris-Cl buffer pH 8.0.

The diafiltered rEPO containing supernatant is applied onto DEAE Sepharose and equilibrated with 10 mM Tris-Cl buffer pH 8.0. The rEPO binds weakly and is recovered in the flow-through while contaminants remain bound to the column. Diafiltration into low-conductivity buffer prior to anion-exchange chromatography ensures stronger binding of contaminants and higher degree of purification at this step. The collected DEAE flow-through is diafiltered into 10 mM sodium malonate buffer pH 6.0 and applied to CM Sepharose equilibrated with the 10 mM sodium malonate pH 6.0 buffer. The rEPO binds to CM Sepharose while contaminants flow through the column. The column is then washed with 10 mM sodium malonate buffer pH 6.0 containing 100 mM NaCl, to further remove contaminants. The elute rEPO from the column, a 10 mM sodium malonate buffer pH 6.0 containing 150 mM NaCl is used.

The eluant containing rEPO is applied to a second CM Sepharose column equilibrated with 10 mM sodium malonate buffer pH 6.0. It is then washed with 10 mM sodium phosphate buffer pH 7.0 and finally, rEPO is eluted in PBS (10 mM sodium phosphate, 150 mM NaCl).

The EPO expressed is glycosylated and has a molecular weight of approximately 25 kD. The amino acid sequence is the same as or analogous to that set forth in literature and patents cited herein. It is quite surprising that the EPO in accordance with the present invention stimulates erythropoiesis as the inventive EPO has glycosylation which does not include sialic acid residues, and there is no O-glycosylation because the EPO is from baculovirus expression; and, any reported recombinant EPO from baculovirus expression heretofore was reported as having no such activity.

In particular, urinary EPO (also known as uEPO) and recombinant EPO produced in mammalian cells are heterogenously glycosylated with complex N- and O-linked oligosaccharides, including sialic acid N-terminal residues, and are acidic proteins, whereas EPO from recombinant baculovirus expression can have a comparably simple saccharide constitution and relative homogeneity, with no sialic acid residues, neutral high-mannose moieties predominating and the highly basic charge density of EPO retained, because of the limited capacity of insect cells to process N-linked oligosaccharides.

Certain literature such as Quelle et al., Blood, supra, at 656, indicates that EPO from expression by insect cells infected with recombinant baculovirus containing DNA coding for EPO is not biologically active due to the lack of sialic acid residues. Further, there is a body of literature asserting that EPO's "heavy glycosylation" and sialic acid residues are essential for biological activity, see, e.g., Marmont, Tumori 83(4 Suppl 2):S3-15 (1997), Morimoto et al., Glycoconj J 13(6):1013-20 (1996), Higuchi et al., J. Biol. Chem. 267(11):7704-9 (Apr. 15, 1992), Takeuchi et al., Glycobiology 1(4):337-46 (1991), Tsuda et al., Eur. J. Biochem. 188(2):405-11 (1990), Takeuchi et al. J. Biol. Chem. 265(21):12127-30 (1990), Fakuda et al., Blood 73(1):84-9 (1989); Matsumoto et al. Plant Mol. Bio. 27(6):1163-72 (1995) (EPO from tobacco cells lacking sialic acid residues lacked activity).

In contrast, the recombinant EPO of the present invention has an activity of at least 200,000 U/mg (indeed about 500,000 U/mg) and stimulates erythropoiesis. In further contrast to prior EPO, the EPO of the present invention can be isolated using anion exchange and cation exchange chromatography, as opposed to reverse chromatography (used for isolating prior EPO).

Thus, the recombinant EPO of the present invention is distinct from and surprisingly superior to prior EPO.--

IN THE CLAIMS:

Kindly add new claims 96-116, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

--96. (New) A substantially pure, recombinant glycosylated erythropoietin, produced by a baculovirus expression system in cultured insect cells, wherein said erythropoietin has relative homogeneity or is purified to 95% or greater and said erythropoietin stimulates erythropoiesis and has an activity of at least 200,000 U/mg or of about 500,000 U/mg.

97. Erythropoietin of claim 96 wherein said erythropoietin stimulates erythropoiesis and has an activity of at least 200,000 U/mg.

98. Erythropoietin of claim 96 wherein said erythropoietin stimulates erythropoiesis and has an activity of at least 500,000 U/mg.

99. (New) Erythropoietin of claim 96 produced by a method comprising:

culturing insect cells in at least one bioreactor whereby there is an insect cell culture,

wherein the insect cells contain a recombinant baculovirus containing exogenous DNA encoding erythropoietin,

supplying medium in at least one vessel whereby there is culture medium,

circulating culture medium and/or insect cell culture, whereby the bioreactor and vessel are in fluid communication and the insect cell culture and/or culture medium are in circulation,

delivering oxygen to the insect cell culture and/or culture medium, and

collecting the expressed product, and/or baculovirus and/or the cells.

100. (New) Erythropoietin of claim 96 produced by a method comprising:

culturing insect cells in a bioreactor whereby there is an insect cell culture,

wherein the insect cells contain a recombinant baculovirus containing exogenous DNA encoding erythropoietin,

supplying culture medium in a vessel whereby there is culture medium,

circulating the insect cell culture through a dialysis means,

circulating culture medium through the dialysis means,

wherein the dialysis means in fluid communication with the bioreactor and the vessel,

whereby

there is

a first, cell culture, loop between the bioreactor and the dialysis means, and

a second, media replenishment, loop between the vessel and the bioreactor,

performing dialysis between the culture medium and the cell culture, and

collecting the erythropoietin.

101. (New) Erythropoietin as claimed in claim 100, wherein the method further comprises:

delivering oxygen into the cell culture loop and measuring physical and/or chemical parameter(s) of the cell culture and/or the culture medium.

102. (New) Erythropoietin as claimed in claim 101, wherein the method further comprises adjusting physical and/or chemical parameter(s) of the cell culture and/or the culture medium in response to data from the measuring.

103. (New) Erythropoietin as claimed in claim 101, wherein the method further comprises measuring pH and measuring dissolved oxygen concentration, adjusting physical and/or chemical parameter(s) of the cell culture and/or the culture medium in response to data from the measuring, wherein the adjusting comprises adjusting temperature to maintain a desired temperature, adjusting pH to maintain a desired pH, and adjusting dissolved oxygen concentration and dissolved carbon dioxide concentrations, whereby the dissolved carbon dioxide levels are adjusted in response to pH measurement(s).

104. (New) Erythropoietin as claimed in claim 103, wherein the method further comprises adjusting dissolved oxygen levels in response to dissolved oxygen measurement(s), adjusting pH to a desired level in response to pH measurement(s) by adjusting the dissolved carbon dioxide concentration such that dissolved carbon dioxide concentration is adjusted when pH varies from the desired level, and the dissolved oxygen measurement varies periodically as a function of time, adjusting the dissolved oxygen concentration so that the dissolved oxygen measurement varies from 30% to 90% or from 40% to 80% or from 50% to 70%; or, so that the dissolved oxygen measurement averages about 60%.

105 (New) Erythropoietin as claimed in claim 104, wherein the adjusting of the dissolved oxygen concentration so that the dissolved oxygen measurement varies from 30% to 90%.

106 (New) Erythropoietin as claimed in claim 104, wherein the adjusting of the dissolved oxygen concentration so that the dissolved oxygen measurement varies from 40% to 80%.

107 (New) Erythropoietin as claimed in claim 104, wherein the adjusting of the dissolved oxygen concentration so that the dissolved oxygen measurement varies from 50% to 70%.

108 (New) Erythropoietin as claimed in claim 104, wherein the adjusting of the dissolved oxygen concentration so that the dissolved oxygen measurement averages about 60%.

109. (New) Erythropoietin as claimed in claim 104, wherein the method further comprises adjusting the dissolved oxygen concentration so that the dissolved oxygen measurement varies from high value to low value over about 10 to about 30 minutes or over about 20 minutes.

110. (New) Erythropoietin as claimed in claim 103, wherein the method further comprises adjusting dissolved oxygen levels in response to dissolved oxygen measurement(s), and adjusting pH to a desired level in response to pH measurement(s) by adjusting the dissolved carbon dioxide concentration such that dissolved carbon dioxide concentration is adjusted when pH varies from the desired level, and the dissolved oxygen measurement varies periodically as a function of time, and wherein a plot of the dissolved oxygen measurement as a function of time comprises a sine wave.

111. (New) Erythropoietin as claimed in claim 99 wherein the insect cells are *Spodoptera frugiperda* cells.

112. (New) Erythropoietin as claimed in claim 100 wherein the insect cells are *Spodoptera frugiperda* cells.

113. (New) Erythropoietin as claimed in claim 111 wherein the medium is serum free.

114. (New) Erythropoietin as claimed in claim 112 wherein the medium is serum free.

115. (New) Erythropoietin as claimed in claim 111 wherein the insect cells are *Spodoptera frugiperda* SF900+ cells.

116. (New) Erythropoietin as claimed in claim 112 wherein the insect cells are *Spodoptera frugiperda* SF900+ cells.--

Kindly cancel claims 1-42, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents